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Antibioticos SA
(Spain),
Bravo Murillo 38, Madrid,
Spain
(72) Inventors
Francisco Salto Maldonado,
Luis Costa Pla,
Jose Maria Fernandez Sousa-Faro,
Jose Luis Fernandez Puentes
(74) Agents
F. J. Cleveland and Company,
40—43 Chancery Lane,
London WC2A 1JQ

(54) **A process for the preparation of polyene antifungal antibiotics and new tetraene antifungal antibiotic**

(57) Polyene antifungal antibiotics are produced by fermenting a culture medium containing assimilable carbon and nitrogen sources with the new microorganism named *Streptomyces sp.* strain SF—1 (ASA), NCIB 11738. A new tetraene antifungal antibiotic is prepared by the above process, and also the known antifungal antibiotic Pimaricin.

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SPECIFICATION

A process for the preparation of polyene antifungal antibiotics and new tetraene antifungal antibiotic

This invention relates to a process for the preparation of polyene antifungal antibiotics, some of them unknown up to date, by way of a ferment process of a new microorganism belonging to the *Streptomyces* genus.

It is also an object of this invention to provide a new tetraene antibiotic.

The polyene antibiotics are substances, the fundamental characteristic of which is that they have a structure containing conjugated double bonds conferring very interesting biological properties thereon.

Examples of these compounds are amphotericin B (U.S. Patents 2 908 611 and 2 908 612), the philippine complex (British Patent 783,486), nystatin (British Patent 866,600), pimaricin, also known as tennecetin or natamycin (British Patent 852,883 and German Patent 1,024,206), candicidin (U.S. Patent 2,992,162), etc. These substances may be distinguished from each other by their UV and IR spectra and by chromatography (paper, thin layer or high pressure).

The polyene antibiotics are mainly produced by microorganisms of the *Actinomycetales* order, particularly *Streptomyces* and *Streptoverticillium*.

The biosynthesis of these compounds is not usually effected univocally, but the appearance of several polyenes in one same culture medium is observed.

The action mechanism on the eukaryotic cells is that of acting on the membrane sterols, causing lysis of the cell on altering the selective permeability conferred by these sterols.

The polyenes are widely used in the field of medicine, although due to their toxicity in intravenous administration, they have been mainly used topically or orally, since the toxicity is then practically non-existent.

However, what is making the polyenes to be substances of current interest is the finding that there is a difference between the action on the permeability of the membrane of tumour cells and normal cells. Another interesting fact is that they reduce the blood cholesterol levels when some of these compounds (candicidin) are administered orally. It is also known that certain polyenes act against hypertrophy of the prostate gland. This is why a search was made for microorganisms which produce these substances.

In this invention there is particularly described the production of several polyene substances having antifungal activity, outstanding among which is a new one named Ab 400 by the applicant, the main biological characteristic of which is that of inhibiting the growth of fungi and yeasts, whereby they may be of use in the treatment of infections caused by pathogenic microorganisms (belonging to the classes mentioned), both in animals and plants.

Ab 400 is produced in the culture medium of an aerobic germ belonging to the *Streptomyces* genus, which has been called *Streptomyces sp. strain SF—1 (ASA)*. A further characteristic of this microorganism is the production of other polyene substances, outstanding among which for its amount is the already known tetraene called pimaricin (see above).

There is also described the extraction and identification of these substances, as well as the antifungal spectrum of Ab 400.

The microorganism used has been isolated from a soil sample from the surroundings of Madrid and has been deposited in the collection belonging to ANTIBIOTICOS, S.A. in Leon (Spain) under the name of *Streptomyces sp. strain SF—1 (ASA)* and in the NATIONAL COLLECTION OF INDUSTRIAL BACTERIA (NCIB), Torry Research Station, Aberdeen, Scotland, under No. NCIB 11,738.

The guidelines appearing in "The Actinomycetales" by SYKES and SKINNER (1973) Academic Press were followed for the taxonomic identification of the microorganism and the conclusion was reached that it belongs to the *Streptomyces* genus, since it has an aerial mycelium with sporophores containing multiple spores in chains, which are not verticillate nor in sporangia. Fragmentation of the mycelium has not been observed.

The methods described by SHIRLING and GOTTLIEB in the "International Streptomyces Project" — ISP — (Int. J. Sist. Bacteriology 16:313—40, 1966) were followed for the characterisation of the species. Under the phase contrast microscope, the microorganism SF—1 (ASA) is observed to have helical sporophores with 1 to 6 more or less closed spirals in the ISP—3 and ISP—4 media, with chains of over ten 0.8 x 0.5 μ oval spores. In the ISP—2 and ISP—5 media, these sporophores do not exhibit genuine spirals, but more or less closed hooks which, at times, close up in a spiral are observed. The spores show a verrucose surface under the electron microscope.

The characteristics of the cultures in the different media are given in Table I.

All the cultures were made at 28°C. The colours were designated in two ways, the former following the sheets of the "Code Universal des Couleurs" by E. SEGUY (1936) Ed. P. LECHEVALIER (Paris) and the latter by giving them subjective names, using the regular names for those colours.

The nitrogen (N) source assimilation spectrum is given in Table II. 1% glucose and 1.5% agar (LUEDEMANN G. M. Int. J. Sist. Bacteriol. 21: 240—47 (1971)) were used as base medium.

The carbon (C) source assimilation spectrum was prepared following the method of SHIRLING, E. B. and GOTTLIEB, D. (Int. J. Sist. Bacteriol. 16: 313—340 (1966)). The results are given in Table III.

The physiological characters are given in Table IV.
To sum up, the strain under study has the following taxonomic characters:

TABLE I

Morphological and Physiological Characteristics of the Streptomyces SP. SF–1 (ASA) Cultures

Ref.	Medium name	Time in days	Growth	Aerial mycelium	Substrate mycelium	Soluble pigm.	Comments
1	ISP–2 agar yeast-malt	21	+++	Orange 180 Greyish violet	Red 116 Blackish brown	Strong brown Not pH indicator	Velvety growth. Abundant sporulation
1	ISP–3 agar oatmeal	21	++	Violet 675 Dark Grey violet	Violet 687 Madder maroon	Very light	Velvety growth. Good sporulation
1	ISP–4 agar starch salts	21	++	Violet 675 Dark grey violet	Violet 687 Madder maroon	Sparing	Velvety growth. Fair sporulation
1	ISP–5 agar glycerol asparagine	21	+++	Violet 675 Dark grey violet	Yellow 312 Greeny black	Strong brown Not pH indicator	Velvety growth. Abundant sporulation
3	Bennet Agar with $CaCO_3$ (0.1%)	14	++	Reddish brown 707. Pale purple black	Violet 641 Candle black	Red. Not pH indicator	Velvety growth. Few spores. Increase in number of spores if $CaCO_3$ is added
3	Czapek Agar (Suchrose)	14	+	–	–	–	Very little growth
3	Emerson Agar with $CaCO_3$ (0.1%)	14	++	Cream	Cream	Negative	Grainy growth.
3	Agar glucose aparagine. Beef extract	14	++	Red 41. Reddish brown	Violet 641 Candle black	Positive Red	Slight sporulation

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TABLE I (Continued)

Morphological and Physiological Characteristics of the Streptomyces SP. SF–1 (ASA) Cultures

Ref.	Medium name	Time in days	Growth	Aerial mycelium	Substrate mycelium	Soluble pigm.	Comments
3	Agar glucose yeast extract with phosphate	14	+++	Violet 606 Violet grey	Violet 641 Candle black	Strong Not red colour indicator	Velvety growth
4	Agar thyrosine	14	–	–	–	–	–
5	No. 172 ATCC	14	+			Sparing	Grainy growth. Little sporulation
6	Agar milk	14	+++	Red 87	Red 87	Positive Dark grey	Does not hydrolyse

1. SHIRLING, E. B. and GOTTLIEB, D. Int. J. Sist. Bacteriol. 16: 313–40 (1966).
3. WAKSMAN, S. A. "The Actinomycetes", Vol. 2 (1961).
4. GORDON, R. E. and SMITH, M. M. J. Bacteriol. 69: 147–50 (1955).
5. "American Type Culture Collection", (ATCC), Catalogue, page 518 (1980).
6. LUEDEMANN, G. M., Int. J. Sist. Bacteriol. 21: 240–7 (1971)

TABLE II
ASSIMILATION OF N SOURCES
BY STREPTOMYCES sp. SF—1 (ASA)

	Growth*	Prod. soluble pigment
Control	–	–
NH_4NO_3	–	–
N—Z amine A	+	–
Yeast extract	++	++
Asparagine	+	+++
Glutamic acid	±	–

* 14 days at 28°C.

TABLE III

ASSIMILATION OF C SOURCES
BY STREPTOMYCES sp. SF—1 (ASA)

Carbon Source	Growth*
Control	–
D-glucose	++
Suchrose	±
Inositol	±
D-fructose	++
Raffinose	±
L-arabinose	–
D-xylose	–
D-mannitol	++
L-rhamnose	–
D-galactose	±
D-arabinose	–
α-melibiose	–
Glycerol	++
β-lactose	–
Starch	+
D-ribose	–

TABLE III (continued)

ASSIMILATION OF C SOURCES
BY STREPTOMYCES sp. SF—1 (ASA)

Carbon Source	Growth*
Cellobiose	++
Trehalose	++
Sorbose	—
Sorbitol	—
Mannose	++
Dulcitol	—
Melezitose	—

*21 days at 28°C.

TABLE IV

PHYSIOLOGICAL DATA OF STREPTOMYCES sp. SF—1 (ASA)

Production of melanic pigments	Response	Reference
a) ISP—6	Negative	1
b) ISP—7	Negative**	1
c) ISP—1	Negative	1
Gelatine hydrolysis	Strong	2
Starch hydrolysis	Slight	2
Skimmed milk	negative hydrolysis	3
Nitrates reduction	Positive	4
NaCl resistence	4% is limit	4
Growth at different temperatures	Is mesophylic	

** A strong dark purple brown pigmentation is produced after 14 days.

1. SHIRLING, E. B. and GOTTLIEB, D. Int. J. Sist. Bacteriol. 16: 313—40 (1966).
2. WAKSMAN, S. A. "The Actinomycetes" Vol. 2 (1961). The Williams and Wilkins Company.
3. GORDON, R. E. and SMITH, M. M. J. Bacteriol. 69: 147—50 (1955).
4. LUEDEMANN, G. M. Int. J. Sist. Bacteriol. 21: 240—7 (1971).

TABLE V

Taxonomic Differences among Pimaricin Producing Streptomyces

Phenotype	*St. gilbosporeus* ATCC–13.326 (1)	*St. chattanoogensis* ATTC–13.358(2)	*St. natalensis* NRRL–2.651(3)	*Streptomyces sp.* SF–1 (ASA)(4)
Melanic pigments		negative	negative	negative
Aerial mycelium colour	Red	yellow or white	grey or red	red or violet
Substrate mycelium colour	maroon yellow	not studied	maroon yellow	maroon yellow + red
Soluble pigment	negative	positive yellow	negative	positive red
Sporophore shape	spiral	spiral	spiral	spiral
Spore wall shape		spiny	spiny	verrucose
Nitrate reduction	positive	positive	not studied	positive
NaCl resistance		does not grow 3%		does not grow 5%
Use of carbon source				
a) Xylose		–	++	–
b) Suchrose		++	–	±
c) Inositol		++	++	±

1. British Patent 846,933 (1960)
2. SHIRLING, E. B. and GOTTLIEB, D. Int. J. Sist. Bacteriol. 18: 69—189 (1968)
3. SHIRLING, E. B. and GOTTLIEB, D. Int. J. Sist. Bacteriol. 22: 265—394 (1972)
4. Results obtained by this Laboratory.

a) It belongs to the Spirals section.
b) It has spores with verrucose surface.
c) It does not produce melanoid pigments.
d) The colour of the sporulated aerial mycelium belongs to the violet or red series, according to the medium used.
e) It produces a soluble red (reddish brown) pigment which is not a pH indicator.
f) The colour of the substrate mycelium varies from reddish maroon to violet maroon, according to medium.
g) It assimilates the following as sole carbon source:

— glucose	— glycerol
— inositol	— starch
— fructose	— cellobiose
— mannitol	— trehalose
— galactose	— mannose

It is doubtful whether it assimilates suchrose and raffinose.

h) Other physiological properties are:
1. Produces powerful hydrolysis of gelatine.
2. Reduces nitrates to nitrites
3. Does not hydrolyse milk casein
4. Withstands up to 4% NaCl
5. It is mesophylic

i) It produces several tetraene antifungal antibiotics.

When observing the taxonomic properties of the *Streptomyces* species in "Bergey's Manual of Determinative Bacteriology" (8th Edition 1974, The Williams and Wilkins Company) and in the "International *Streptomyces* Project" papers published by SHIRLING and GOTTLIEB in "Cooperative description of Type strains of *Streptomyces*" (Int. J. Sist. Bacteriol. 18:69—189, 1968; ibid 18: 279—392, 1968; ibid 19: 391—512, 1969, and ibid 22: 265—394, 1972), as well as those published from 1974 to date, no microorganism similar to SF—1 (ASA) has been found.

It was compared particularly with those *Streptomyces* which also produce pimaricin, namely *S. natalensis, S. chattanoogensis* and *S. gilbosporeus.* The results of this study are given in Table V and lead to the conclusion that *Streptomyces sp. SF—1 (ASA)* is different from the other pimaricin producers.

The microorganism described herein is the wild strain, but the mutants thereof, obtained both spontaneously and induced by mutagenic agents, may also produce the group of polyene antibiotics together or separately, whereby it is understood that in the process described hereinafter, both the wild strain and the mutant descendents thereof may be used.

PRODUCTION BY FERMENTATION

The tetraenes described herein may be produced in solid or liquid media, although the latter are more appropriate for the preparation of large amounts of the compounds. In the liquid media, the antibiotics may be produced both by surface and submerged culture, the latter being the best for preparing large amounts of product.

The production medium must comprise at least:

a) an assimilatable carbon source such as glucose, fructose, mannitol, glycerol, starch, dextrin, cellobiose, trehalose, mannose, alone or in combination.
b) an assimilatable nitrogen source such as vegetable cake meals (soy, cotton, ground nut, etc.), corn steep liquor, vegetable or animal peptones, yeast, urea, ammonia salts, etc, alone or in combination.
c) Mineral salts to provide the medium with a pH buffering power or to supply mineral elements.
d) Vegetable or animal oils to be used as foam suppressors and also as carbon source. Silicones.

The pH fermentation range allowing for production is broad, from 5.1 to 8.4 The optimum temperature lies between 25 and 30°C.

The regular antibiotic fermentation processes are used to prepare the tetraenes of the invention by fermentation, namely, inclined tubes containing a medium in which the strain sporulates well are seeded with lyophilised spores, they are incubated at the optimum temperature and the growth phase in submerged liquid culture is started with the spores and mycelium from these tubes. These stages will be longer or shorter and more or less numerous depending on the broth volume to be fermented. If large fermentation tanks are used, the number of stages may reach 5.

To ferment in a flask, it is sufficient to shake on the appropriate shaking table in a thermostat

controlled chamber; when fermenting in tank, this must have aerating, stirring, pH control, dissolved O_2 control, temperature control and nutrient, alkali and acid addition systems for the production to reach an optimum level. All the processes must be carried out under absolute sterile conditions, to avoid contamination with extraneous microorganisms. The media may be sterilised in the vessels or be placed therein already sterilised, provided that the vessel has been previously sterilised.

Production starts once there is sufficient mycelium in the medium, which happens as from 48 hours; this production increases gradually up to the 5th or 6th day of fermentation, the increase then ceasing.

To study the amount of antibiotic in the broth, as a standard for Ab 400 there was used a purified sample which showed a single active substance in paper and high pressure liquid chromatography (HPLC) and which, against *Saccharomyces cerevisiae ATCC 9767* had a minimum inhibiting concentration of 0.5 μg/ml. As a pimaricin standard, there was used a sample provided by KONINKLIJKE NEDERLANDSCHE GIST- & SPIRITUSFABRIEK N.V. of Delft, Holland, (Batch 9117 A) of 900 U/mg.

Although the existence of Ab 400 in the broths of the microorganisms already patented was not described, a comparative study was performed on *Streptomyces sp SF—1 (ASA)* and *St. natalensis* NRRL—2651 and *St. chattanoogensis* ATCC 13,358 to find out whether this substance was produced in the fermentation in the media described in the patents and in one of those used herein. The results are given in TABLE VI. Here the clear differences between the already patented pimaricin producing strains and that of the invention, since while *Streptomyces sp. SF—1 (ASA)* produces a high amount of Ab 400, as well as pimaricin, in all the media tested, there was no detectable production of the former in the two strains mentioned. A further differentiating feature of the SF—1 (ASA) strain is the production of a strong apple smell in the fermentation broths.

TABLE VI

PRODUCTION OF Ab 400 AND PIMARICIN
BY ST. NATALENSIS, ST. CHATTANOOGENSIS AND ST. SP. SF—1 (ASA)

Microorganism	Fermentation medium	mcg/ml Pimaricin	mcg/ml Ab 400
S. Sp. SF—1 (ASA)	SFMF—3*	288	409
	MCH—1*	61	92
	MFNa—1*	73	205
S. natalensis NRRL 2651	SFMF—3	26	0
	MCH—1	322	0
	MFNa—1	32	0
S. chattanoogensis ATCC 13.358	SFMF—3	10	0
	MCH—1	76	0
	MFNa—1	127	0

* The SFMF—3 medium contains 4% soy bean meal, 6% glucose, 0.5% $(NH_4)_2SO_4$; 0.13% $K_2HPO_4.3H_2O$; 1% $CaCO_3$ and 0.5% soy bean oil, pH 6.2.

The medium known as MCH—1 is described in British Patent 852,883 and contains 2% glycerol, 0.5% phytone, 0.5% peptone; 0.3% yeast extract, 0.3% meat extract and 0.25% $CaCO_3$. pH 7.6.

The medium known as MFNa—1 is described in German Patent 1 024 206 under the letter D and comprises 5% soy bean meal; 1% glucose; 0.5% $(NH_4)_2SO_4$; 0.02% KH_2PO_4; 1% $CaCO_3$; 0.5% soy bean oil and 0.1% corn steep liquor pH 6.3.

EXTRACTION AND ISOLATION

Once fermentation has finished, the minimum inhibitory concentration (MIC) of the filtered broth is normally 1/2000 against *Saccharomyces cerevisiae*.

The whole broth, the pH of which ranges from 5 to 6 is filtered through Hyflo-Supercell and the filtered broth is adjusted to pH 8.0 by sodium hydroxide followed by extraction with a non-water miscible aliphatic alcohol, such as butanol.

The organic extract is concentrated under vacuum to reduce the volume to 1/20 of the original volume, whereby a white powder is precipitated out and which has been called the "raw antifungal". This is filtered and dried under vacuum at room temperature. The MIC of this very slightly yellow powder is around 1/1,500,000 (0.67 mcg/ml) against the above-mentioned yeast. The yield of this product is 0.7 g per litre of filtered broth.

The "raw antifungal" is relatively water-soluble and in it there are at least two main antibiotics which are active against *Saccharomyces cerevisiae*, as checked by paper chromatography.

If the "raw antifungal" is dissolved in water at a rate of about 4 mg/ml and this solution is adjusted to pH 10.2 and allowed to rest at +1° (for 24 hours) it precipitates a white substance having a MIC of 1/10,000,000 (0.1 mcg/ml) known as "antifungal 1" and corresponding to the said Ab 400. This antifungal 1 or Ab 400 is obtained with a yield of 6.6% of the starting "raw antifungal".

The ultraviolet spectrum of "antifungal 1" in methanol shows that it is a tetraene and the infrared spectrum in potassium bromide is similar, although not identical, to the tetraene antifungal pimaricin.

Pimaricin has a lower R_f than the "antifungal 1" in a butanol + ethanol + water (5:4:1, v) chromatographic system.

Using high pressure liquid chromatography (HPLC) with a LiCrosorb rp-18 column (5 u), a mobile phase of 0.06 M ammonium citrate, pH 5.0 + acetonitrile (3:1, v/v) and detection at 300 nm, 7 substances having antibiotic activity against *Saccharomyces cerevisiae* and with tetraene ultraviolet spectra were identified in the "raw antifungal".

The elementary centisimal composition of the "antifungal 1" was C 54.5%, H 7.1%, N 2.9%, O (dif.) 35.5%, giving a minimum empirical formula of $C_{22}H_{34}O_{11}N$ and, consequently, a minimum molecular weight of 488,522.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of Ab 400 was studied. There was no activity against bacteria and a positive activity against yeasts:

Candida	albicans
"	anomala
"	dolonii
"	desusei
"	melinii
"	pelliculosa
"	pulcherrima
"	tropicalis
Cryptococcus	albidus
"	difluens
"	neoformans
Debaryonyces	hansenii
"	klockenii
Geotricum	versiforme
Hansenula	pseudopelliculosa
"	suaveolens

Pichia pseudopolimorfa

" membranaefaciens

The following Examples are provided to provide a better illustration of the invention.

EXAMPLE 1

A medium was prepared with the following composition:

Cotton seed meal	20 g
Corn steep liquor	10 g
Dextrin	10 g
Glucose	50 g
$(NH_4)_2SO_4$	5 g
$NaNO_3$	2,5 g
K_2HPO_4	1 g
$FeSO_4 . 7 H_2O$	0,05 g
$MgSO_4 . 7H_2O$	0,05 g
$MnSO_4 . H_2O$	0,05 g
$ZnSO_4 . 7H_2O$	0,1 g
Distilled water ad	1000 ml

The pH is adjusted with KOH to 6.2.

200 ml portions of the medium are placed in 1,000 ml Erlenmeyers.

1 g of $CaCO_3$ was added to each flask.

The flasks were sterilised at 121°C for 30 minutes.

The thus prepared medium was seeded with a *Streptomyces sp.* strain SF—1 (ASA) spore suspension. This suspension was prepared from an inclined tube culture in any solid medium in which the microorganism may sporulate.

After seeding, this medium was incubated for 5 days in a chamber controlled by thermostat to 28°C under constant shaking on an orbital shaking table with a 5 cm stroke and a speed of 240 r.p.m. The thus prepared innoculum may be used to seed the fermentation medium, which comprises the following composition.

Soy bean meal	40 g
Glucose	60 g
$(NH_4)_2SO_4$	5 g
K_2HPO_4	13 g
$CaCO_3$	10 g
Distilled water ad	1000 ml

The pH was adjusted to 6.2 with dilute sulphuric acid before adding the carbonate.

200 ml portions of sodium were placed in 1 litre flasks.

1 ml of soy bean oil was added to each flask.

The flasks were sterilised at 121°C for 30 minutes.

After sterilisation, the medium was seeded with 5% of the innoculum.

This was incubated for 4 days at 28°C in a chamber, on a shaking table of the same characteristics as described for the innoculum. The analysis of the broth at the end of the fermentation gives:

pH 5.5—6.5; sugar 2%; ammonium N: 100 mg/l;
total soluble N: 950 mg/l and mycelial volume: 32.

Against *Sacch. cerevisiae* ATCC 9767 it has a minimum inhibitory concentration of 1/4,000 to 1/5,000; in HPLC it shows an Ab 400 activity of 300—500 mcg/ml and a pimaricin activity of 1,000 to 1,200 mcg/ml, using pimaricin of 900 U/mg as a standard.

EXAMPLE 2

A medium was prepared with the same composition as the innoculum of Example 1 and it was placed at the rate of 200 ml medium in 1 litre Erlenmeyers. It was sterilised at 121°C for 30 minutes. The thus prepared medium was seeded with a suspension of spores of the microorganism of the patent. It was incubated for 5 days at 28°C in a thermostat controlled chamber with constant shaking on an orbital shaking table having a 5 cm stroke and a shaking speed of 240 r.p.m.

The thus prepared innoculum was used to seed fermenting tanks of 8 litre capacity containing 4 litres of medium having the same composition as the fermentation medium of Example 1, at a rate of 0.5%. The fermentation conditions were: temperature 28°C, aeration 0.3 v/v/m, stirring 400 r.p.m., fermentation time 90 hours. At that time the fermentation medium had a pimaricin activity of 500—1000 mcg/ml and an Ab activity of 300—600 mcg/ml. The same type of standards were used as for Example 1.

EXAMPLE 3

3,540 ml of whole broth, at pH 5.9, were filtered under vacuum through a prelayer of Hyflo-Supercell to provide 2,150 ml of reddish clear filtered broth with MIC = 1/2,222. The pH was adjusted to 8.0 with 25% sodium hydroxide and the flocculent precipitate was filtered off in the same way and rejected. A first extraction was made of the filtered broth with 700 ml of butanol by centrifuging the organic phase. The aqueous phase was extracted a further two times with 350 ml of butanol each time, in the same way as above. The total clear yellow butanol extract was concentrated under vacuum to 1/20 of the initial volume causing precipitation of a white powder which, after filtering, washing with butanol and drying under vacuum at room temperature weighed 1.52 g; namely, 0,71 g per litre of filtered broth. This "raw antifungal" showed a MIC = 1/1,450,000 (0.69 mcg/ml).

The product was dissolved in 380 ml of water and the pH was adjusted to 10.2 with sodium hydroxide. After 24 hours at +°C, the flocculent precipitate was filtered, washed with water and dried under vacuum at room temperature to provide 101 mg of antifungal 1; 6.7% of the starting "raw antifungal". The MIC of this antifungal 1 was 1/10,000,000 (0.1 mcg/ml).

EXAMPLE 4

3,800 ml of whole broth, at pH 5.6, were filtered under vacuum on Hyflo-Supercell, to provide 2,500 ml of filtered broth with MIC = 1/2000. The pH was adjusted to 8.0 with sodium hydroxide and the broth was refiltered in a similar way. It was extracted with butanol as in the previous example and the butanol extract was concentrated under vacuum to 1/20 of the initial volume. The precipitated "raw antifungal" was filtered, washed with butanol and dried under vacuum at room temperature, to provide 2.0 g having a MIC = 1/1,666,000 (0.60 mcg/ml).

The above raw antifungal was dissolved in 500 ml and the solution pH was adjusted to 10.2 with sodium hydroxide, the solution being stored for 24 hours at +1°C. The precipitated antifungal 1, after drying, weighed 131 mg; 6.6%. The MIC was 1/10,000,000 (0.1 mcg/ml).

EXAMPLE 5

3,750 ml of whole broth, at pH 5.9, after filtering over Hyflo-Supercell, gave 2,300 ml of filtered broth, with MIC = 2,500. This was adjusted to pH 8.0, filtered, extracted with butanol in the same way as in previous Examples. After concentration of the organic extract, 1.9 g of raw antifungal with MIC = 1/2,222,000 (0.45 mcg/ml) were obtained. The raw antifungal, after dissolution in 475 ml of water, pH 10.2, gave 146 mg of antifungal 1, 7.7%, MIC 1/10,000,000 (0.1 mcg/ml) after 24 hours at +1°C.

CLAIMS

1. A process for the preparation of polyene antifungal antibiotics wherein a fermentation of a culture medium with the microorganism named Streptomyces sp. strain SF—1 (ASA) is carried out, said antibiotics being extracted from the mycelium and/or from the culture medium.

2. The process of claim 1, wherein said fermentation is aerobic, advantageously at a temperature of from 25°C to 30°C and the culture medium contains assimilatable carbon and nitrogen sources and mineral salts.

3. The process of claim 2, wherein there is used as carbon source glucose, fructose, mannitol, glycerol, starch, dextrin, cellobiose, trehalose, mannose, alone or combinations thereof and there is used as nitrogen source vegetable cake meals, corn steep liquor, vegetable or animal peptones, yeast urea, ammonium salts alone or combinations thereof.

4. The process of any one of claims 1 to 3, wherein the resulting fermentation broth is filtered, the

pH is adjusted to approximately 8.0, the moisture is extracted and it is concentrated under vacuum, thereby precipitating out an antibiotic complex or raw antifungal, from which pimaricin is isolated.

5. The process of any one of claims 1 to 3, wherein the resulting fermentation broth is filtered, the pH is adjusted to approximately 8.0, the moisture is extracted and it is concentrated under vacuum, thereby precipitating out an antibiotic complex or raw antifungal, which is dissolved in water at a rate of about 4 mg/ml, the solution pH is adjusted to approximately 10.2 and subsequently there is provided by precipitation a tetraene antibiotic named Ab 400.

6. A tetraene antifungal antibiotic which may be produced by the process of claim 5, having an elementary centesimal composition of C—54.5%; H—7.1%; N—2.9%; O—35.5% and a minimum inhibiting concentration of 1/10,000,000 against Saccharomyces cerevisiae.

7. Streptomyces sp. strain SF—1 (ASA) identified by No. NCIB 11738.

8. Polyene antifungal antibiotics substantially as hereinbefore described.

9. A method of preparing polyene antifungal antibiotics substantially as hereinbefore described.

10. Polyene antifungal antibiotics whenever produced by the method claimed in any one of claims 1 to 5.

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